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Abstract: Molecular communication in biology is mediated by protein interactions. According to the current paradigm, the specificity and affinity required for these interactions are encoded in the precise complementarity of binding interfaces. Even proteins that are disordered under physiological conditions or that contain large unstructured regions commonly interact with well-structured binding sites on other biomolecules. Here we demonstrate the existence of an unexpected interaction mechanism: the two intrinsically disordered human proteins histone H1 and its nuclear chaperone prothymosin- associate in a complex with picomolar affinity, but fully retain their structural disorder, long-range flexibility and highly dynamic character. On the basis of closely integrated experiments and molecular simulations, we show that the interaction can be explained by the large opposite net charge of the two proteins, without requiring defined binding sites or interactions between specific individual residues. Proteome-wide sequence analysis suggests that this interaction mechanism may be abundant in eukaryotes.

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Extreme disorder in an ultra-high-affinity protein complex

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Summary

Molecular communication in biology is mediated by protein interactions. According to the current paradigm, the specificity and affinity required for these interactions are encoded in the precise complementarity of binding interfaces. Even proteins that are disordered under physiological conditions or contain large unstructured regions commonly interact with well-structured binding sites on other biomolecules. Here we demonstrate the existence of an unexpected interaction mechanism: The two intrinsically disordered human proteins histone H1 and its nuclear chaperone prothymosin α associate in a complex with picomolar affinity, but they fully retain their structural disorder, long-range flexibility, and highly dynamic character. Based on the close integration of experiments and molecular simulations, we show that the interaction can be explained by the large opposite net charge of the two proteins without requiring defined binding sites or interactions between specific individual residues. Proteome-wide sequence analysis suggests that this interaction mechanism may be surprisingly abundant in eukaryotes.

In the conventional paradigm of structural biology, intermolecular interactions are encoded in the complementary shapes and noncovalent forces between folded biomolecules. However, it has become increasingly clear that many proteins involved in cellular interactions are fully or partially unstructured under physiological conditions[1,2]. In some cases, these intrinsically disordered proteins (IDPs) form a well-defined 3D-structure upon target binding[1]; in others, parts of the complex remain disordered. A broad spectrum of such protein complexes with different degrees of disorder are known[3]: Sometimes, a well-defined and structured binding interface is formed in the bound state, and only some loops or the chain termini stay disordered. In other cases, one of the binding partners remains almost completely unstructured in the complex, and its multiple binding motifs dynamically interact with the folded partner. Examples include interdomain interactions in the cystic fibrosis transmembrane regulator[4]; the cyclin-dependent kinase inhibitor Sic1 binding to the substrate recognition subunit of its ubiquitin ligase subunit Cdc4[5]; the tail of human Na^+/H^+ exchanger 1 with the extracellular signal-regulated kinase ERK2[6]; or nuclear transport receptors interacting with nucleoporins[7]. The underlying multivalent binding enables unique regulatory mechanisms[8] and can mediate the formation of liquid-liquid phase separation[9], indicating the emergence of new modes of biomolecular interactions.

We have discovered a pair of proteins that constitutes an extreme case of a highly unstructured protein complex with physiological function. One binding partner, the linker histone H1.0 (H1), which is involved in chromatin condensation by binding to nucleosomes[10,11], is largely unstructured[12] and highly positively charged, with two disordered regions flanking a small folded globular domain (Fig. 1, Extended Data Table 1). The other partner, the abundant nuclear protein prothymosin α (ProTα), is a fully unstructured, highly negatively charged IDP[13,14] involved in chromatin remodeling[15], transcription, cellular proliferation, and apoptosis[16]. ProTα acts as a linker histone chaperone by interacting with H1 and increasing its mobility in the nucleus[17]. We show here that ProTα and H1 bind to each other with very high affinity, but both proteins fully retain their structural disorder. Based on the integration of complementary experimental techniques and molecular simulations, we obtain a detailed model of this highly disordered and dynamic protein complex, which represents a new paradigm of biomolecular binding.

A highly unstructured protein complex

The binding of H1 to ProTα has been demonstrated both *in vitro*[18] and *in vivo*[17]. However, their high net charge, low hydrophobicity, and pronounced disorder in the free proteins raise the question of how much structure is formed when they interact. We used circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy to investigate the formation of secondary and tertiary structure. The CD spectra of unbound ProTα and H1 reflect the low secondary structure content of the individual IDPs, except for the small helix-turn-helix domain of H1[13,19,20] (Fig. 1c). Surprisingly, the CD spectrum of an equimolar mixture of the two proteins can be explained by the simple sum of the individual spectra, indicating that complex formation entails minimal changes in average secondary structure content.

To obtain residue-specific information, we employed NMR spectroscopy. $^1H,^{15}N$ heteronuclear single quantum coherence (HSQC) spectra of the individual proteins exhibit low dispersion of the ^1H chemical shifts, as expected for IDPs[14,21-23] (Fig. 1e,f). Only the globular domain of H1, which is stably folded even in isolation (Extended Data Fig. 1), shows the large dispersion of resonances characteristic of tertiary structure[23,24] (Fig. 1g). Remarkably, the overall peak dispersion remains unchanged upon complex formation, confirming that no pronounced tertiary structure is formed upon binding. Nevertheless, small but clearly detectable peak shifts observed for ProTα and H1 indicate significant changes in the average chemical environment of the corresponding residues, as expected upon interaction with the large opposite charge of the other IDP. For ProTα, 95% of the amide backbone nuclei could be assigned (Extended Data Fig. 2), enabling a residue-specific analysis: The C^α secondary chemical shifts[25] of ProTα show no evidence for the induction of persistent or transiently populated secondary structure upon complex formation (Fig. 1d). The severe overlap in the NMR spectra of the unstructured parts of H1 precluded residue-specific assignments, but the clusters of H^α-C^α peaks in the $^1H,^{13}C$-HSQC spectrum from the lysine-rich disordered regions neither exhibit detectable chemical shift perturbations upon titration with

ProTα, nor do additional resonances emerge (Extended Data Fig. 3e,f). We thus have no indications of changes in secondary structure content in H1 upon ProTα binding.

The lower intensity of the resonances corresponding to the H1 globular domain (Fig. 1f,g, Extended Data Fig. 3) is likely to originate from the faster transverse (T_2) relaxation of structured compared to unstructured regions; additionally, tumbling of the globular domain is decelerated by the drag of the unstructured regions it is embedded in[26]. Upon complex formation, the intensity of many H1 (and ProTα) resonances decreases, and those of the globular domain drop below the noise (Extended Data Fig. 3b and Fig. 1f,g). The large hydrodynamic radii of H1 and the complex (Extended Data Fig. 4a,b) support a large effective rotational correlation time as the origin of peak broadening, but a contribution from chemical exchange cannot be excluded. Note, however, that the globular domain is dispensable for complex formation (Fig. 2b, cf. *High-affinity binding in spite of disorder*).

High-affinity binding in spite of disorder

To quantify the strength of the interaction between H1 and ProTα, we used single-molecule Förster resonance energy transfer (FRET), which enables measurements over a very broad range of affinities, down to the picomolar regime. By labeling two positions with a donor and an acceptor dye, distances and distance changes between or within the polypeptides can be determined by confocal fluorescence detection of molecules freely diffusing in solution[27,28]. ProTα labeled at positions 56 and 110 (ProTα 56C-110C) exhibits a mean transfer efficiency, $\langle E \rangle$, of 0.33 at near-physiological ionic strength (Fig. 2a, Extended Data Table 2), as expected for this IDP, which is highly expanded owing to its large negative net charge[13,29,30]. Upon addition of unlabeled H1, a population with higher $\langle E \rangle$ of 0.58 (i.e. shorter average distance) emerges: Evidently, binding the positively charged H1 leads to a compaction of ProTα by charge screening, analogous to that obtained upon addition of salt[29]. The same behavior is observed for doubly labeled H1 (Extended Data Table 2), demonstrating a mutual adaptation of the conformational ensembles. The resulting dissociation constant in the low picomolar range reveals an extremely strong interaction (Fig. 2b, Extended Data Table 2), consistent with the physiological role of ProTα as a linker histone chaperone[17] competing with the tight binding of H1 to chromatin[31]. Measurements with other FRET dyes and label positions resulted in similar affinities (Extended Data Table 2), indicating that labeling has only a small effect on binding. The dominant contribution to the interaction with ProTα stems from the unstructured C-terminal part of H1, which alone binds with picomolar affinity. The N-terminal half and the isolated globular domain of H1 also bind ProTα, but with much lower affinity (Fig. 2b). At least four isolated globular domains can bind to one ProTα molecule at the same time, with modest chemical shift changes (Extended Data Fig. 1), suggesting the absence of a specific binding interface.

The large and opposite net charges of ProTα (-44) and H1 (+53) imply a strong electrostatic contribution to binding. Indeed, a mere doubling of the ionic strength from the physiological 165 mM to 340 mM reduces the affinity by six orders of magnitude (Fig. 2c). By extrapolation, a reduction of ionic strength to ~140 mM would take this interaction into the femtomolar range. From low picomolar to 100 μM protein concentrations, the stoichiometry from intermolecular FRET (Extended Data Fig. 4c) and NMR chemical shift titrations (Extended Data Figs. 2 and 3), as well as the hydrodynamic radii measured with pulsed-field gradient NMR and two-focus fluorescence correlation spectroscopy (2f-FCS) (Extended Data Fig. 4a,b) indicate the predominant formation of one-to-one dimers and the absence of large oligomers or coacervates[32]. However, in the presence of a large excess of one of the binding partners, a decrease in FRET efficiencies is indicative of the weak association of additional molecules with a K_D in the 10 to 100 μM range (Extended Data Fig. 4d,e), a propensity also observed in the simulations described below.

A highly dynamic complex

The lack of structure formation in the H1-ProTα complex implies great flexibility and a highly dynamic interconversion within a large ensemble of configurations and relative arrangements of the two IDPs. The presence of a broad, rapidly sampled distance distribution is supported by the analysis of fluorescence

lifetimes[28,33,34] (Extended Data Fig. 5). Since fluctuations in distance cause fluctuations in the fluorescence intensity of donor and acceptor, the timescale of these long-range distance dynamics can be measured by single-molecule FRET combined with nanosecond fluorescence correlation spectroscopy (nsFCS)[34,35]. For individual unfolded or disordered proteins, reconfiguration times (inter-dye distance relaxation times) between ~20 ns and ~200 ns have been observed[27]. ProTα alone, with its highly expanded chain[13,29] and corresponding lack of impeding intramolecular interactions[36], is a particularly dynamic IDP and yields reconfiguration times, τ_r, between 29^{+2}_{-2} ns and 78^{+15}_{-9} ns, depending on the chain segment probed[34,36] (Extended Data Table 2). H1 (labeled at positions 113 and 194) reconfigures more slowly, with $\tau_r = 118^{+24}_{-14}$ ns, but within the range previously observed for unfolded and disordered proteins[27,34].

Strikingly, these pronounced and rapid long-range dynamics are retained in the complex, with values of τ_r between 66^{+2}_{-2} ns and 191^{+22}_{-19} ns for 13 different labeling pairs throughout the dimer (Fig. 3a-d, Extended Data Table 2). The similarity of τ_r for the two proteins in the complex suggests a coupling of the dynamics of the two intertwining chains. The highly dynamic nature of the complex is further supported by NMR: The longitudinal (T_1) and transverse (T_2) ^{15}N relaxation times reflect rapid backbone dynamics in the pico- to nanosecond range, both for free ProTα and in the complex (Fig. 3h, Extended Data Fig. 2). The increase in T_1/T_2 (Fig. 3h) and R_H (Extended Data Fig. 4), and the reduced peak intensities (Fig. 3f) are consistent with the increase in τ_r for ProTα observed by nsFCS in the complex (Fig. 3a), where chain-chain interactions are expected to moderate both local and long-range dynamics.

Architecture of an unstructured protein complex

To develop a structural representation of the conformational ensemble of the H1-ProTα complex, we combine single-molecule FRET, NMR, and molecular simulations. We first mapped the complex with single-molecule FRET by probing a total of 28 intra- and intermolecular distances with donor and acceptor dyes in specific positions (Figs. 3i, 4a). The resulting intermolecular transfer efficiencies lack pronounced patterns that would be expected for persistent site-specific interactions or chain alignment in a preferred register. The intermolecular transfer efficiencies are most sensitive to the labeling position on ProTα, with the highest efficiencies (i.e. shortest average distances) for the central position ProTα 56, intermediate efficiencies for ProTα 110, and lowest efficiencies (i.e. longest distances) for ProTα 2. These results indicate that the region of highest charge density of ProTα (Fig. 1b) most strongly attracts H1. The charge density along H1 is more uniform (Fig. 1a), as are the transfer efficiencies to ProTα, albeit with some decrease towards the termini (Fig. 3i).

Based on this information, we sought to establish a molecular model of the H1-ProTα complex. Given the lack of structure formation and residue-specific interactions, the dominance of electrostatics, and the size of the system, we used a simplified model in which each residue is coarse grained into a single bead. Coulombic interactions between all charged residues are included explicitly, with a screening factor to account for an ionic strength of 165 mM. Other attractive interactions and excluded volume repulsion are captured via a short-range potential, with the radius of the residues determined from their volumes[37]. A structure-based potential[38] is used to describe the folded globular domain of H1. The transfer efficiencies computed from Langevin dynamics simulations can be matched to the measured values (Fig. 4a) via the single adjustable parameter in our model, namely the contact energy of the short-range potential, which is the same for all residues (see Methods); explicitly including a representation of the chromophores in the simulations yielded very similar results (Fig. 4a). The resulting intra- and intermolecular distance distributions (Extended Data Fig. 6d) are smooth and unimodal, in accord with the absence of site-specific interactions and structure formation observed experimentally, and attesting to the convergence of the simulations. The good agreement between the transfer efficiencies from experiment and simulation indicates that this simple model captures the essential properties of the structural ensemble. Considering its simplicity, the femtomolar affinity estimated from the model (Extended Data Fig. 5b) is remarkably consistent with the affinities observed experimentally near this ionic strength. The affinity for a second molecule of H1 or ProTα to the complex is predicted to be orders of magnitude weaker, consistent with experiment (Extended Data Figs. 4d,e and 6b).

The resulting intra- and intermolecular distance maps (Fig. 4b) indicate that the interactions between ProTα and H1 are broadly distributed along their sequences, but they also reflect the asymmetry in electrostatic attraction owing to the higher charge density of ProTα in its central and C-terminal regions (Figs. 1b, 4a). The NMR results provide an independent experimental test of the model: Indeed, the distribution of the average number of contacts made by the residues of ProTα based on the simulation (Fig. 3e) is strikingly similar to the distribution of changes in chemical shifts, peak intensities, and T_1/T_2 ratios observed upon binding (Fig. 3f-h). These changes occur across the same broad region between residues 46 and 106, encompassing the most acidic tracts of ProTα. Overlap within the Glu cluster prevents the quantitative analysis of some peaks, but similar chemical shift and intensity perturbations as for the rest of the region are observed (Fig. 3f,g).

Further analysis of the simulated structural ensemble (see Supplementary Video) shows a lack of distinct conformational clusters (Extended Data Fig. 6a), implying a continuous distribution of configurations. A projection of the simulation onto the first three principal components of the inter-residue distances (Extended Data Fig. 6c) reveals a highly heterogeneous ensemble of arrangements of the two entwining flexible chains (Fig. 4c). Given the rapid intramolecular dynamics and lack of structure in the complex, the activation barrier for binding is likely to be close to zero. Indeed, association of H1 and ProTα occurs at the diffusion limit, with a binding rate coefficient of $(3.1 \pm 0.1)\cdot 10^9$ $M^{-1}s^{-1}$ (Extended Data Fig. 7). The simulations support this mechanism, with a downhill free energy surface for binding, and attractive fly-casting[39] interactions enhanced by electrostatics[40] emerging already at a distance of ~22 nm, much greater than the sum of the hydrodynamic radii (Extended Data Fig. 6b).

Conclusions

Our results suggest that high-affinity complex formation between two oppositely charged IDPs is possible without the formation of structure or the need for folded domains. In contrast to the prevalent paradigm of molecular recognition in biomolecular interactions, this type of highly dynamic complex neither requires structurally defined binding sites nor specific persistent interactions between individual residues. Rather, the results are well described by long-range electrostatic attraction between the two interpenetrating polypeptide chains, especially between their charge-rich regions. The exceedingly rapid interconversion of many different arrangements and configurations on the 100-ns timescale results in efficient averaging and essentially a mean-field-type interaction[41,42] between all charges. This type of complex expands the known spectrum of protein-protein interactions. Although the complex of H1 and ProTα is extreme in its extent of disorder for both binding partners, the possibility of this interaction mechanism may not be entirely unexpected, given the prevalence of charged amino acids in many IDPs[2], the previous observation of disorder in IDPs interacting with folded proteins[3-7], and the role of electrostatics in the formation of dynamic binding interfaces between folded proteins[43]. Moreover, the H1-ProTα interaction resembles polyelectrolyte complexes formed by charged synthetic polymers[42], even though the latter usually phase-separate into coacervates. The absence of coacervation[32,42] or liquid-liquid phase separation[9] for ProTα and H1 at concentrations from picomolar to high micromolar may be due to the complementarity[44] of the two proteins in terms of effective length and opposite net charge, leading to optimal, mutually saturating electrostatic interactions, or the lack of hydrophobic and aromatic side chains and cation-π interactions, which have been suggested to favor phase separation mediated by proteins[32,45,46].

What are the functional implications of such a high-affinity yet unstructured dynamic complex between two IDPs? Histone H1 is a key factor in chromatin condensation and transcriptional regulation[11], and ProTα acts as a chaperone of H1 that facilitates its displacement from and deposition onto chromatin[17]. ProTα thus needs to be able to compete with the very high affinity of the histone to chromatin[31]. However, high affinities between structured biomolecules are usually linked to exceedingly slow dissociation[40], incompatible with fast regulation. By contrast, the high affinity of the H1-ProTα complex is facilitated by its ultra-fast association, which enables dissociation on a biologically relevant timescale in spite of the high affinity required for function. Another consequence of polyelectrolyte interactions is the possibility of ternary complex formation[47], signs of which are detected here with a large excess of ProTα or H1 (Extended Data Figs. 4d,e and 6b), resulting in mostly unexplored kinetic

mechanisms that cannot be explained by competition via simple dissociation and re-association[48]. Finally, the flexibility within such unstructured complexes may facilitate access for enzymes adding posttranslational modifications, which play key roles in the regulation of cellular processes, including those of H1. One example of this mechanism may be the interaction of the acidic domain of the oncogene SET with the lysine-rich C-terminal tail of p53, which is regulated by acetylation[49].

The behavior we observe for ProTα and H1 might be surprisingly widespread, since highly charged protein sequences that could form such complexes are abundant in eukaryotes. In the human proteome alone, several hundred proteins that are predicted to be intrinsically disordered[50] contain contiguous stretches of at least 50 residues with a fractional net charge similar to that of H1 or ProTα. Since the interaction of highly oppositely charged IDPs is unlikely to be very sequence-specific[18], achieving binding selectivity may be linked to other regulatory mechanisms, e.g. cellular localization or synchronized expression during relevant stages of development or the cell cycle.

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Author contributions

A.B., M.B.B., K.B., B.B.K., R.B.B., and B.S. designed and supervised research; M.B.B., A.B., V.K., and A.Sot. produced and labeled fluorescent protein variants; A.B. and M.B.B. performed single-molecule experiments; A.B., M.B.B., A.Sor., and D.N. analyzed single-molecule data; D.N. developed single-molecule instrumentation and data analysis tools; A.Sot. and A.B. carried out stopped-flow measurements, A.B., M.B.B., K.J.B., and A.Sot. established experimental conditions for single-molecule measurements; C.B.F., and P.O.H. produced protein samples for NMR; K.B. and C.B.F. performed and analyzed NMR measurements; A.Sor. carried out the bioinformatics analysis; R.B.B. conducted and analyzed simulations; A.B., B.B.K. and C.B.F. carried out CD experiments; B.S., A.B., R.B.B., B.B.K. and K.B. wrote the paper with the help from all authors.

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Figure Legends

Figure 1. ProTα and H1 remain unstructured upon binding. Extended configurations of H1 **(a)** and ProTα **(b)**, net charges, and surface electrostatic potentials with color scale (units in k_BT/e). For the globular domain of H1, only residues with a solvent-accessible surface area (SASA) > 0.5 nm^2 are included and indicated by a blue shaded area (cf. Extended Data Table 1). **(c)** Far-UV CD spectra of ProTα (red), H1 (blue), the ProTα-H1 mixture (purple), and their calculated sum (black) at 5 μM for each protein; curves are the mean of n=60 individual spectra, n=2 repeats of this measurement yielded consistent results. **(d)** C^α secondary chemical shifts ($SCS_{C\alpha}$) of ProTα free (red), in complex with H1 (purple), and their differences (black). **(e)** ^1H,^{15}N-HSQC spectra of ^{15}N-ProTα in the absence (red) and presence (purple) of unlabeled H1; n=5 repeats of this measurement yielded consistent results. **(f)** ^{15}N-H1 in the absence (blue) and presence (purple) of unlabeled ProTα (n=2) with zooms (①,②). **(g)** H1 spectra from (f) at lower contour level.

Figure 2. ProTα and H1 form an electrostatically driven high-affinity complex. (a) Single-molecule transfer efficiency histograms of FRET-labeled ProTα 56C-110C without (top) and with increasing concentrations of unlabeled H1 as indicated in the panels, fitted with two peaks, unbound (red) and bound (purple). **(b)** Binding isotherms based on transfer efficiency histograms for full-length H1 (●, $K_D = 2.1^{+1.1}_{-0.8}$ pM), N- (●, $K_D = 173^{+29}_{-28}$ nM) and C-terminal (●, $K_D = 40^{+6}_{-4}$ pM) regions, and the globular domain of H1 (●, $K_D = 1.9^{+0.3}_{-0.3}$ μM) at 165 mM ionic strength (see Extended Data Table 1 for details). **(c)** K_D of H1-ProTα complex as a function of ionic strength with fit[51] (purple line) and 95% confidence interval (shaded). See Methods for details on statistics and data analysis.

Figure 3. Dynamics, interactions, and distances in the complex. (a-d) Examples of nsFCS probing long-range dynamics based on intra- and intermolecular FRET (see Extended Data Table 2 for details); curves are the averages of n=3 independent measurements. **(e)** Average number of contacts of each ProTα residue with H1 based on the simulations (Fig. 4b). **(f)** Ratios of NMR resonance intensities of ProTα in the presence (I) and absence (I_0) of H1. **(g)** Weighted backbone amide chemical shift perturbations (CSPs) of ProTα induced by equimolar H1 binding (see Extended Data Fig. 2 for other stoichiometries); n=5 repeats of this measurement yielded consistent results. In (f,g), the grey horizontal lines represent the average of three unassigned but traceable Glu residues in the range 62-67 with error bars from their standard deviation (see Methods for details). **(h)** Ratios of longitudinal (T_1) and transverse (T_2) ^{15}N relaxation times of ProTα in the free (red) and bound (purple) states (see Extended Data Fig. 2 for details). Light grey stars indicate prolines and unassigned residues, dark grey stars resonance overlap and/or insufficient data quality. Circles are mean values from n=3 consecutive measurements, errors are s.d. The dashed box indicates the sequence range with the largest changes. **(i)** Transfer efficiency (E) histograms from intermolecular single-molecule FRET experiments between different positions in acceptor-labeled ProTα and donor-labeled H1, fitted with a single peak (purple, $\langle E \rangle$ shown). The signal at $E \approx 0$ originates from molecules without active FRET acceptor. For further information on statistics see Methods.

Figure 4. Architecture of the complex from simulations. (a) Comparison of experimental (filled squares) and simulated transfer efficiencies (empty symbols) in the H1-ProTα complex for the pairs of dye positions indicated below (triangles and circles: simulations with and without explicit chromophores, respectively). **(b)** Intra- and intermolecular average distance maps of H1 and ProTα from the simulations, separately and in the complex. The white dashed square indicates the globular domain (only surface-exposed residues shown, see Extended Data Table 1). **(c)** Examples of configurations of H1 (blue) and ProTα (red) in the complex; N-termini are indicated by small spheres. The structures are projected onto the first three principal components (PC) of the distance map, with projections of the full ensemble shown as gray scatter plots (units of Å, see also Extended Data Fig. 6 and Supplementary Video). Numbers indicate the positions of the structures in the PC projections.

Legends Extended Data Figures and Tables

Extended Data Figure 1. Titrations of ProTα and Globular Domain (GD). (a) Titration of ^{15}N-ProTα with 0- to 7-fold molar addition of GD followed by ^{1}H,^{15}N-HSQC spectra; n=2 repeats of this measurement yielded consistent results. **(b)** Peak intensity ratios for assigned residues of ProTα relative to the free state induced by 0- to 1.7-fold molar addition of GD (n=2). **(c)** CSPs per residue of ProTα induced by 0- to 7-fold molar addition of GD (n=2). For comparison, CSPs of ProTα upon 1-fold molar addition of H1 are shown in grey (n=5). Panels **a-c** follow color key 1; grey stars indicate prolines and unassigned residues. **(d)** ProTα CSPs plotted against concentration and times excess of GD relative to the free state for residues 46-106 upon 0- to 7-fold molar addition of GD. Curves corresponding to individual residues are shown in different colors for clarity. **(e)** Far-UV CD spectrum of GD. **(f)** Thermal denaturation of GD followed by the change in ellipticity at 222 nm (T_m = 320.5 ± 0.3 K, ΔH_m = -44 ± 2 kcal mol^{-1}). Inset: Fraction of unfolded GD (f_u) as a function of temperature. **(g)** Titration of 100 μM ^{13}C,^{15}N-GD with 0- to 7-fold molar addition of ProTα followed by ^{1}H,^{15}N-HSQC spectra (color key 2). Peak intensities gradually decrease during the titration. At 3.5×- and 7× excess ProTα, natural abundance peaks of free ProTα appear (^{1}H,^{15}N-HSQC spectrum of ^{15}N-ProTα shown in grey for comparison). **(h)** CSPs of GD plotted against concentration and times excess of ProTα relative to the free state upon 0- to 7-fold molar addition of ProTα. A total of 66 (unassigned) amide backbone peaks were followed and grouped according to the standard deviation (STD) of the CSPs (1 STD = 0.0254 ppm). Of these, 55% had CSPs larger than 1 STD.

Extended Data Figure 2. Titration of ^{15}N-ProTα with H1. (a) ^{1}H,^{15}N-HSQC spectrum of 11 μM free ^{15}N-ProTα with assigned residues labeled (left) and titrated with 0- to 4-fold molar addition of H1 (right) (see color key); n=5 individual repeats of this measurement yielded consistent results. **(b)** Weighted backbone amide chemical shift perturbations (CSPs) of ProTα (residues 46-106) relative to the free state upon 0- to 4-fold molar addition of H1, plotted against concentration and times excess of H1. Curves corresponding to individual residues are shown in different colors for clarity. **(c)** CSPs and **(d)** peak intensity ratios for assigned residues of ProTα induced by 0- to 4-fold molar addition of H1 (for bar colors, see key); n=5 for both. **(e)** Longitudinal ^{15}N relaxation times (T_1) of free (red) and H1-bound (purple) ^{15}N-ProTα. $\langle T_1 \rangle$ is 610 ms (free) and 636 ms (complex); n=2 individual repeats of this measurement yielded consistent results.

(f) Transverse ^{15}N relaxation times (T_2) of free (red) and H1-bound (purple) ^{15}N-ProTα. $\langle T_2 \rangle$ is 302 ms (free) and 217 ms (complex). In c-f, light grey stars indicate prolines and unassigned residues, dark grey stars overlap and/or insufficient data quality. Circles in e and f are mean values from n=3 consecutive data acquisitions on the same samples, errors are s.d.

Extended Data Figure 3. Titration of ^{13}C,^{15}N-H1 with ProTα. (a) ^{1}H,^{15}N-HSQC spectra of free ^{13}C,^{15}N-GD (globular domain, dark green) and free ^{13}C,^{15}N-H1 (orange). The majority of the amide peaks of the GD overlap with the more dispersed peaks from full-length H1, indicating the similarity in structure of the GD in isolation and within H1. **(b)** Titration followed by ^{1}H,^{15}N-HSQC spectra of ^{13}C,^{15}N-H1 with 0- to 4-fold molar addition of ProTα. Data acquired on His_6-tagged H1; n=2 individual repeats of this measurement yielded consistent results. **(c)** CSPs relative to free H1 of eleven traceable H1 amide backbone peaks from the intrinsically disordered region (based on overlay with ^{1}H,^{15}N-HSQC spectra of GD (a)) upon 0 to 4-fold molar addition of ProTα plotted against concentration and times excess. Curves corresponding to individual residues are shown in different colors for clarity. **(d)** CSPs plotted against peak intensity ratios relative to the free state of H1 of the eleven H1 amides at 1× excess of ProTα. Colors as in (c). **(e)** Overlay of the C^{α},H^{α} region from ^{1}H,^{13}C-HSQC spectra of free ^{13}C,^{15}N-H1 (blue) and ^{13}C,^{15}N-GD (green). The H1 ^{1}H,^{13}C-HSQC spectrum is dominated by intense clusters of peaks not present in the GD spectrum, consistent with the large fraction of residue repeats in the H1 disordered regions. **(f)** C^{α},H^{α} region of ^{13}C,^{15}N-H1 upon titration with ProTα. The lack of detectable changes in C^{α},H^{α} resonances

is consistent with the absence of secondary structure induction in the disordered regions of H1 upon binding.

Extended Data Figure 4. Hydrodynamic radii and stoichiometry of the H1-ProTα complex. (a) Hydrodynamic radii, R_H, of free and bound ^{15}N-ProTα (100 μM) determined with pulsed-field gradient NMR at 283 K. The signal decays of free ^{15}N-ProTα (red), with H1 at a 1:1 molar ratio (purple), and with H1 GD at a 1:7 molar ratio (green) as a function of gradient strength, together with corresponding fits and a table of the diffusion coefficients and resulting R_H values. **(b)** R_H measured by 2f-FCS at 295 K. Lines show the mean R_H from n=2 independent measurements of H1 -1C (blue) and ProTα 2C (red) labeled with Alexa 594 in the absence of binding partner. Symbols represent the mean R_H from n=2 independent measurements of labeled ProTα (5 nM) in the presence of equimolar concentrations of unlabeled ProTα and unlabeled H1. S.d. is indicated by error bars or shaded bands. **(c)** Stoichiometry ratio[71] versus transfer efficiency plots from intermolecular single-molecule FRET measurements of ProTα 2C + H1 194C (top), ProTα 56C + H1 194C (middle), and ProTα 110C + H1 194C (bottom, variants labeled as indicated in the panels); a stoichiometry ratio of 0.5 indicates a 1:1 complex. The peaks at $E \approx 0$ originate from molecules or complexes lacking an acceptor dye. **(d,e)** Transfer efficiency changes at large excess of unlabeled binding partner for FRET-labeled ProTα 56C–110C (d) and H1 104C–194C (e). See Methods for further information on statistics.

Extended Data Figure 5. Fluorescence lifetime analysis. Plots of the fluorescence lifetimes of donor (Alexa 488), τ_D^D, and acceptor (Alexa 594), τ_D^A, normalized by the intrinsic donor lifetime, τ_D^0, versus the ratiometric transfer efficiency, E (calculated from the number of donor and acceptor photon counts), as a diagnostic for the presence of a broad distance distribution rapidly sampled during the time of a fluorescence burst[28,33,34]. If fluctuations in transfer efficiency occur on a timescale between the donor fluorescence lifetime (~4 ns) and the burst duration (~1 ms), the normalized donor lifetimes cluster above, and the acceptor lifetimes below the solid diagonal line expected for a single fixed distance, as previously observed for intrinsically disordered proteins[34,72]. The large deviation from the diagonal observed for both unbound and bound ProTα and H1 supports the presence of broad, rapidly sampled distance distributions. **a.** ProTα 56C–110C. **b.** ProTα 56C–110C + unlabeled H1. **c.** H1 -1C–194C. **d.** H1 -1C–194C + unlabelled ProTα. **e.** ProTα 2C + H1 194C. **f.** ProTα 110C + H1 194C. All variants labeled as indicated by the cartoons in the figure panels.

Extended Data Figure 6. Simulation results. (a) Decision graph using the Rodriguez-Laio clustering algorithm[70], showing only a single density maximum distant from other density maxima, i.e. a single distinct cluster. **(b)** Free energy of association for ProTα and H1 from simulation, yielding a K_D of 7 fM at an average inter-protein distance of $R_{PH} = 0$ (black curve). Blue and red curves are the free energies for addition of a second H1 or a second ProTα, respectively, to an existing H1-ProTα complex. **(c)** Principal component (PC) vectors shown as contact maps. Colors indicate the increase or decrease in each pair distance for that PC, relative to the other distances. ProTα and H1 residue numbers are indicated in red and blue, respectively. Each PC describes a feature of the chain arrangement: PC_1, e.g., captures the presence or absence of interactions between the ProTα N-terminus and H1. **(d)** Intramolecular (top row) and intermolecular (rows 2 to 4) distributions of distances corresponding to FRET labeling sites, within the ProTα-H1 complex (labels P*X*-H*Y* refer to residues *X* and *Y* in ProTα and H1, respectively). Filled distributions: simulations without explicit chromophores; green lines: simulations with explicit chromophores.

Extended Data Figure 7. Kinetics of H1-ProTα binding measured by stopped flow. FRET-labeled ProTα 56-110 was mixed rapidly with unlabeled H1 in TBS buffer, and the resulting increase in acceptor fluorescence was monitored (inset, example at 10 nM H1 with single-exponential fit and residuals above,

see Methods for details). Decay rates were obtained from single-exponential fits, with an instrument dead time of 3 ms. Standard errors for each H1 concentration were obtained via bootstrapping. The observed rates, k_{obs}, are shown as a function of H1 concentration (c_{H1}); for H1 concentrations between 10 and 100 nM, where pseudo-first order conditions apply (ProTα concentration after mixing was 2 nM), they were fit with $k_{obs} = k_{on}c_{H1} + k_{off} = k_{on}c_{H1} + k_{on}K_D$, using the independently determined K_D of 2.1 pM (Extended Data Table 2). The fit yields a bimolecular association rate coefficient of $k_{on} = (3.1 \pm 0.1)\cdot 10^9$ M^{-1} s^{-1} and an apparent dissociation rate coefficient of $k_{off} = (6.5 \pm 3.1)\cdot 10^{-3}$ s^{-1}. The gray area represents the 95% confidence band.

Extended Data Figure 8. Example of the quality of the H1 preparation. Electrospray ionization mass spectrum of H1 T161C labeled with Alexa 488 (calculated mass 21,800 Da) and reversed-phase HPLC (Vydac C4) chromatogram (inset) showing absorption at 280 nm (red) and 488 nm (blue) and the elution gradient from solvent A (5% acetonitrile in H_2O + 0.1% TFA) to solvent B (100% acetonitrile) (black), illustrating the high purity of the sample. The peak at ~5.5 min corresponds to free Alexa 488, the peak at ~16.8 min to H1 T161C labeled with Alexa 488.

Extended Data Table 1. Sequences of protein constructs and fluorescently labeled variants of H1 and ProTα. (top) Sequences of H1 and ProTα wildtype and variants used. Bold yellow-shaded residues are positions mutated to Cys for fluorophore conjugation. Residues in red are part of protease recognition sites used to cleave the HisTag with thrombin (GGPR or GC) or HRV-3C (GP). (Note that the wt sequence of H1 starts with "T"; the preceding Cys residue (-1) was added for labeling.) The underlined H1 sequence indicates the globular domain (GD), identified based on a sequence alignment with the *G. gallus* homolog[20] (PDB access code 1HST, 82% sequence identity). Surface-exposed residues (as shown in Fig. 1a and 5b) are shaded in light blue. The net charge of each variant is indicated in parentheses. [a]C-terminal disordered region. [b]N-terminal disordered region including GD. **(bottom)** Labeled variants of H1 and ProTα. [c]Förster radius of the corresponding dye pair.

Extended Data Table 2. Binding affinities, molecular dimensions, and reconfiguration times of fluorescently labeled H1 and ProTα. (top left) Affinities of labeled ProTα for H1 at different ionic strength (IS) and for H1 fragments for 165 mM IS ([b]see Extended Data Table 1). Uncertainties for the IS dependence are standard errors estimated from two independent titrations ([a]uncertainty at 165 mM: see Methods), for fragment binding from dilution errors (see Methods). [c]Apparent K_D from fraction of all bound species. **(top center)** Binding affinities of ProTα and H1 labeled with different dye pairs for the respective unlabeled partner. [d]Uncertainties based on dilution errors. **(top right)** Transfer efficiencies and average distances of ProTα and H1 labeled with different dye pairs in the bound (R_{bound}) and unbound state ($R_{unbound}$). Uncertainties in distance are based on an estimated systematic error of ± 0.05 in the transfer efficiency from instrument calibration for the different dye pairs. **(bottom left)** Intermolecular reconfiguration times for the complex of donor-labeled H1 and acceptor-labeled ProTα and *vice versa*. **(bottom right)** Reconfiguration times of doubly labeled ProTα and H1 (unbound and bound). Uncertainties estimated by propagating the error on the transfer efficiency (± 0.05).

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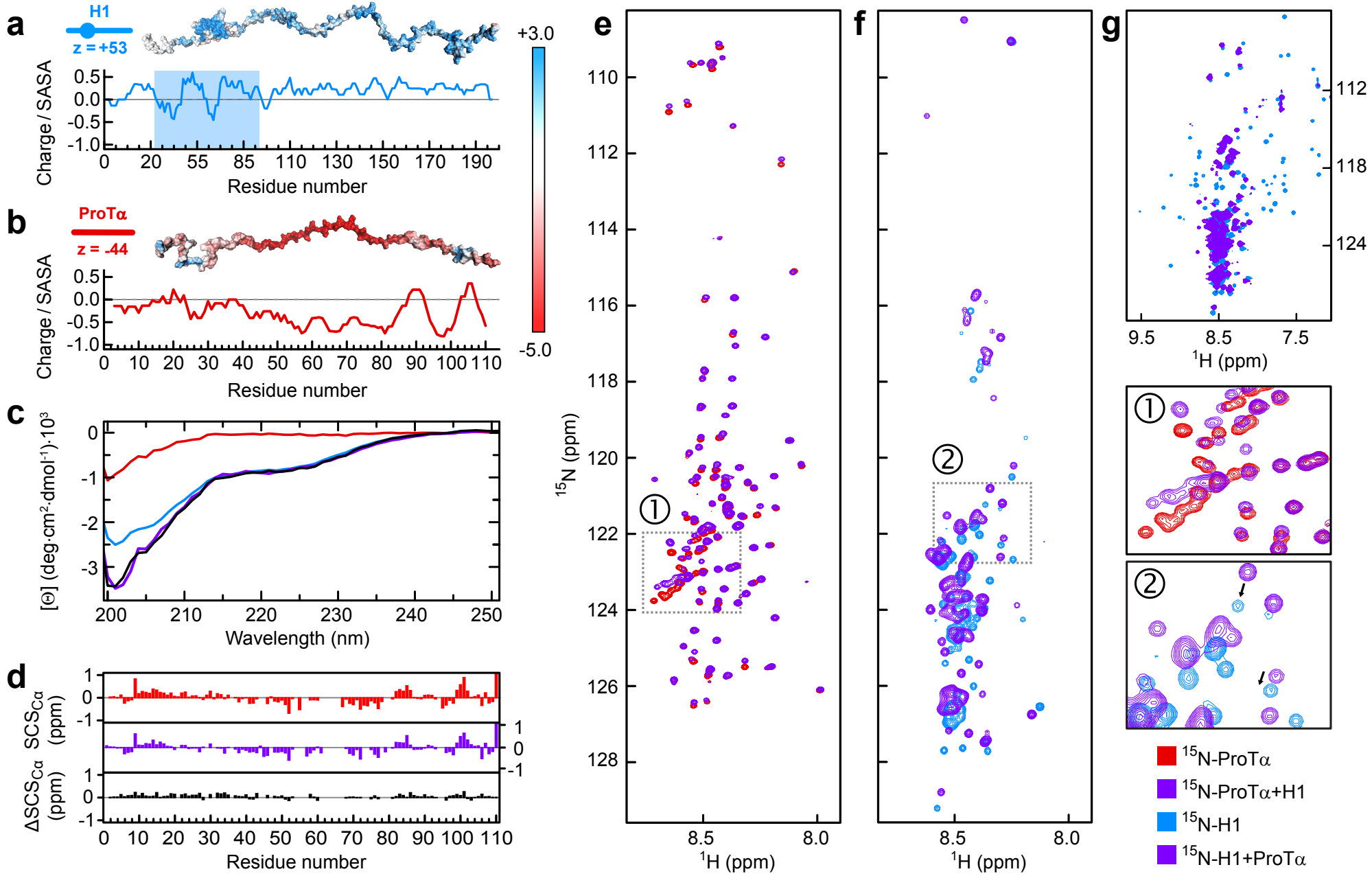
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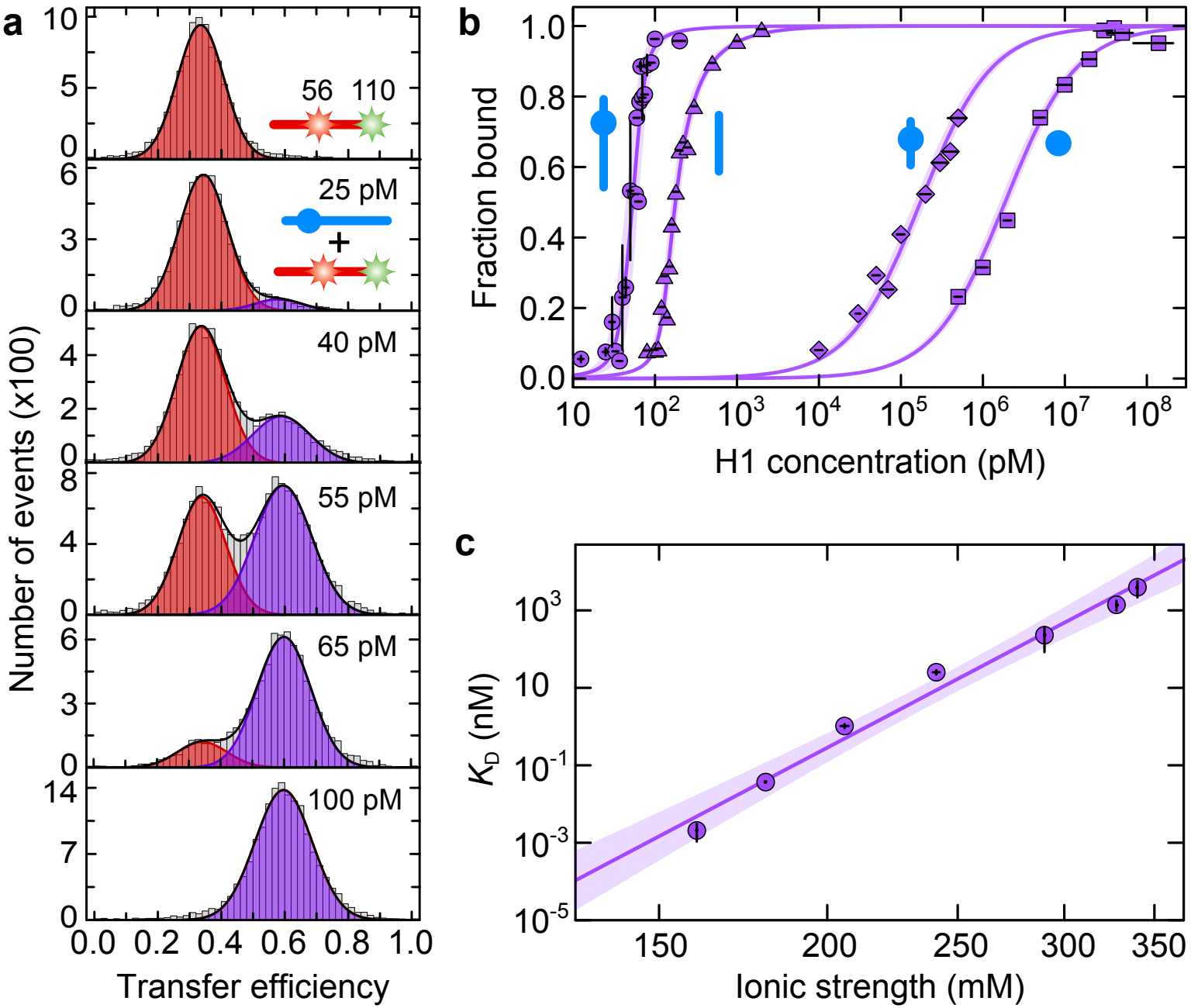
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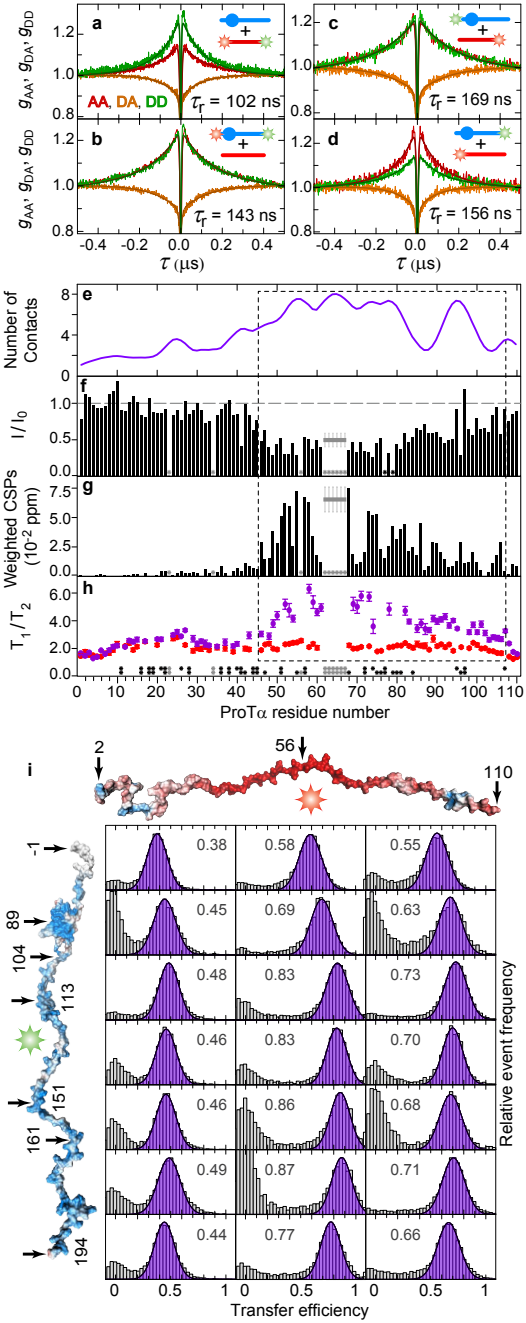
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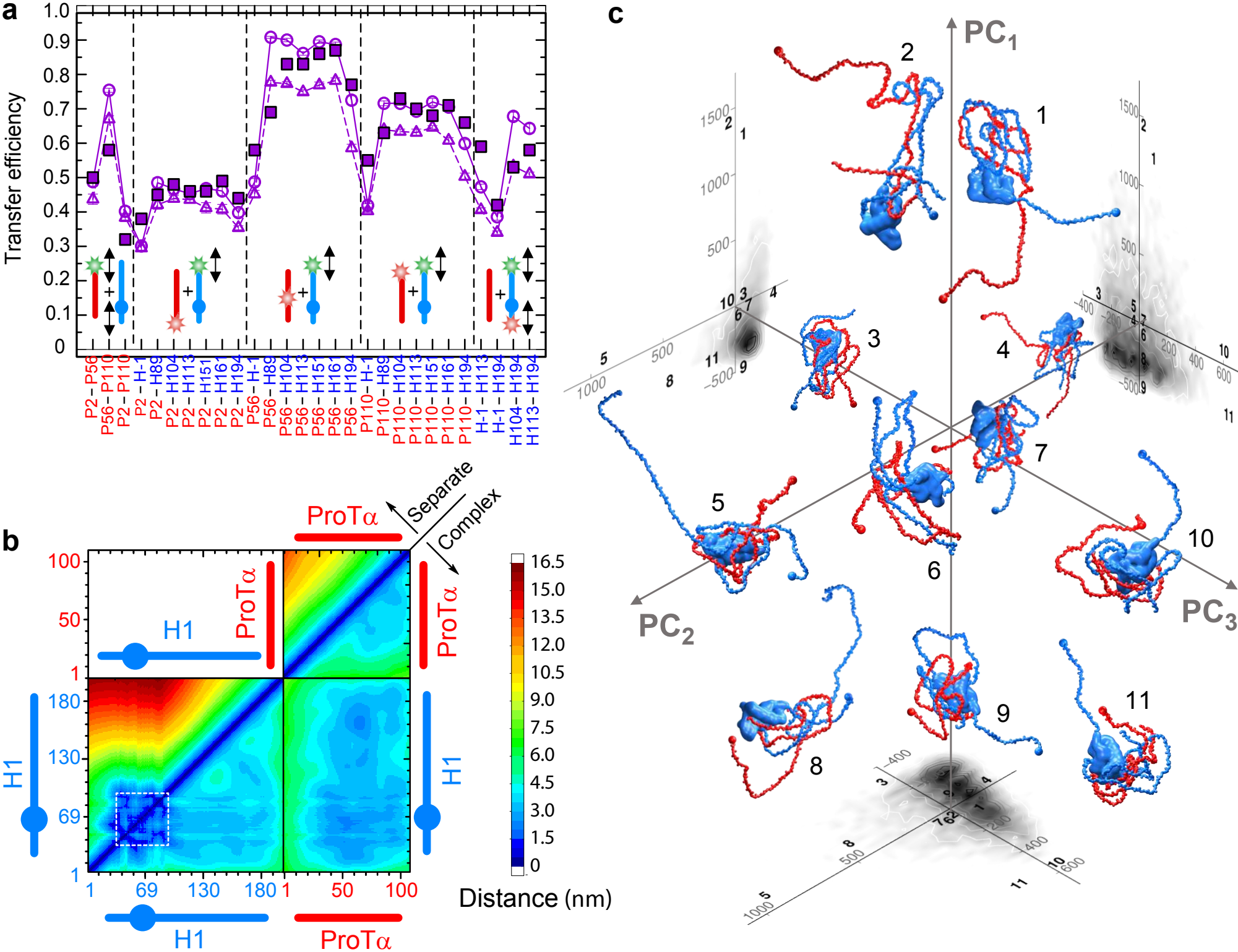
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